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Ketone bodies, glycolysis, and K_{ATP} channels in the mechanism of the ketogenic diet

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Summary

The ketogenic diet (KD) has shown remarkable efficacy in the treatment of drug-resistant childhood epilepsy. Our understanding of how the KD produces its anticonvulsant and anti-epileptogenic effects remains incomplete, which is perhaps not surprising for a biological manipulation as sweeping as dietary change. Several hypotheses focus on ketone bodies, fuel molecules that circulate at millimolar concentrations in the blood of patients on a KD, as causative agents. Here I consider some recent evidence for one such hypothesis, involving a possible role for altered glycolysis and consequent activation of a class of potassium channels called K_{ATP} channels.

Keywords

ketogenic diet; K_{ATP} channels; glycolysis; ketones

Various mechanisms have been hypothesized for the anticonvulsant effect of the KD, differing in their focus on one or another of the many metabolic changes likely to occur in patients on the diet (Schwartzkroin, 1999; Bough & Rho, 2007). Many of these hypotheses focus on ketone bodies as crucial mediators of the beneficial effects of the KD. In a patient or animal on a KD, the liver metabolizes fats to acetoacetate, a four-carbon β-keto acid. Acetoacetate is enzymatically reduced to R-β-hydroxybutyric acid, and these two so-called "ketone bodies" circulate at millimolar levels in the blood, and can serve as an energy source to all extrahepatic tissues including the brain. Acetoacetate can spontaneously decarboxylate to produce acetone, which produces the characteristic breath odor of those in ketosis. In most studies, some level of ketosis (elevation of blood ketone bodies) seems to be important for diet efficacy, though there is not necessarily a correlation between the level of ketone bodies and the degree of seizure control.

Some hypotheses for the role of ketone bodies focus on the possibility of their direct pharmacologic effects (Likhodii et al. 2003), while others focus on their role as metabolic fuels (Yudkoff et al. 2007; Ma et al. 2007). *In vitro* studies of ketone bodies as pharmacologic anticonvulsants have been disappointing (Thio et al. 2000), though at high concentrations, acetone and acetoacetate can have acute anticonvulsant effects *in vivo* (Rho et al. 2002; Likhodii et al. 2003). An alternative role for ketone bodies – not as drugs but as metabolic fuels – is supported by early work on the metabolic effects of fasting in humans. Owen et al. (1967) showed that in fasted humans, the brain can extract ketone bodies from

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blood sufficient for ~65% of its energy utilization, with a corresponding reduction in the utilization of glucose. The brain's ability to extract and use ketone bodies depends on monocarboxylate transporters in the blood-brain barrier and on specific enzymes in brain mitochondria, both of which can be induced by fasting or a ketogenic diet.

Rapid reversal of anticonvulsant protection suggests a fast physiological mechanism

The therapeutic effects of a KD for epilepsy can be apparent quite quickly or may require days. This variation may be consistent with the need for induction of the transporters and enzymes required for ketone body utilization. On the other hand, in human patients the reversal of seizure protection upon breaking a KD can be nearly immediate – after eating a candy bar or upon IV infusion of glucose (Huttenlocher, 1976), seizure activity in patients prone to frequent seizures can recur within the hour.

This rapid reversal suggests an important role for acute physiological processes in the anticonvulsant effect of the KD. Upon glucose infusion, the levels of circulating ketone bodies decline on a fast time scale of tens of minutes (Huttenlocher, 1976; except that brain acetone requires many hours to fall; Jones, 2000). Slower mechanisms involving changes in gene and protein expression (Sullivan et al. 2004; Garriga-Canut et al. 2006; Bough et al. 2006) may be important in other therapeutic effects of the KD on epileptogenesis or neuroprotection, but the anticonvulsant effect of the KD appears to involve rapidly responsive mechanisms.

K_{ATP} channels can couple metabolism and excitability

A well-known mechanism for rapid coupling between metabolism and electrical excitability is a class of potassium channels sensitive to ATP: the K_{ATP} channels. These channels play a key role in regulating insulin secretion from pancreatic beta cells. Between meals, the activity of these channels maintains the membrane potential of beta cells at a negative value; upon ingesting a carbohydrate-rich meal, the consequent elevation of intracellular ATP leads these channels to close, allowing action potential firing that promotes insulin secretion.

K_{ATP} channels are also present in many central neurons, but their role is less well understood. Mice with a knockout of the Kir6.2 pore-forming subunit of K_{ATP} channels exhibit increased sensitivity to anoxia-induced seizures (Yamada et al. 2001), implying that (at least under this condition of extreme metabolic stress) K_{ATP} channels can help to prevent excessive and harmful neuronal firing.

Although whole-brain ATP levels are elevated in animals on the KD, the oxidative metabolism of ketone bodies causes a reduction in brain glucose utilization (De Vivo et al. 1978). It has been argued that ATP production from glycolysis (the first phase of glucose utilization) plays a privileged role in controlling processes at the plasma membrane, including the regulation of K_{ATP} channels and the fueling of the ATP-driven Na^+ pumps (see refs in Ma et al. 2007). We therefore considered the possibility that when a brain slice metabolizes ketone bodies, the reduction in glycolytic ATP production might enable the opening of K_{ATP} channels and thus could reduce the electrical excitability of central neurons.

For our experiments, we examined the regular spontaneous firing activity of neurons in the substantia nigra *pars reticulata* (SNpr) (Ma et al. 2007). The GABAergic projection neurons of SNpr contain a high density of K_{ATP} channels, and the SNpr is thought to act as a 'seizure gate' that regulates seizure threshold even for seizures that originate elsewhere in the brain

(Iadarola & Gale, 1982; McNamara et al. 1984; Depaulis et al. 1994; Velišková & Moshé, 2006). We used brain slices from suckling mice or rats (postnatal day 13–15) because these animals are adapted to high fat mother's milk that approximates a KD, so the cells have all of the metabolic machinery needed to metabolize ketone bodies (Nehlig, 1999).

We found that supplying ketone bodies (acetoacetate or R-β-hydroxybutyrate) to these brain slices, in the constant presence of glucose, produced a ~15% reduction in the spontaneous firing rate, over 15–30 minutes of exposure. This small effect became roughly twice as big for faster firing cells, either for cells that naturally fired at a higher rate, or for cells that were stimulated to fire by lowering extracellular [Ca^{2+}]. This "use dependence" – i.e., a larger effect on faster-firing cells – is a common attribute of successful therapeutic interventions for epilepsy, as it allows for a minimal effect on normal brain activity but a strong suppression of epileptiform activity.

These effects of ketone bodies on action potential firing are eliminated in the presence of K_{ATP} blockers, or when the Kir6.2 subunit is knocked out genetically. The data support the idea that K_{ATP} channels are activated in the presence of ketone bodies, but the mechanism of this activation is not yet clear. The time course seems too slow for a direct pharmacologic effect of the ketone bodies on the channels, but it is consistent with a change in cellular metabolism.

ATP compartmentation – the notion that glycolytic ATP production is especially critical for plasma membrane processes – would explain the basic effect that ketone bodies tend to activate K_{ATP} channels, as glycolysis is reduced when ketone bodies are metabolized. It could also explain the apparent use-dependence, as increased firing allows more Na^+ to enter the cell. The Na^+ must be pumped out by the plasma membrane Na^+ pump, thus consuming ATP in the same submembrane compartment where ATP is produced by glycolysis and sensed by K_{ATP} channels. Indeed, there is evidence from brainstem respiratory neurons that firing activity can provoke K_{ATP} channel opening by a mechanism involving the Na^+ pump (Haller et al. 2001).

K_{ATP} channels may thus be part of a normal negative-feedback mechanism – and perhaps a built-in seizure protection mechanism – whose set-point is determined by the level of glycolysis and can be manipulated by ketone body metabolism. Such a mechanism, if it does operate, is likely not restricted to the SNpr, as many central neurons express K_{ATP} channels. Preliminary evidence on hippocampal dentate granule neurons suggests that K_{ATP} channel open probability is increased by action potential firing, and that both this induced opening and the basal open probability are augmented in the presence of ketone bodies (G. Tanner and G. Yellen, unpublished).

Under this K_{ATP}-glycolysis hypothesis, the key effect of ketone bodies is to reduce glycolytic activity. Reduced glycolysis is also an apparent trigger step for certain changes in gene expression that can retard epileptogenesis (Garriga-Canut et al. 2006). In principle, glycolysis can be reduced either by inhibiting it with a compound like 2-deoxyglucose, by lowering glucose, or by providing an oxidative substrate (like ketone bodies). The advantage of providing an oxidative substrate is that it permits a switch to an alternative fuel source without compromising the option to use glycolysis in case of metabolic stress.

It is not known exactly how glucose and ketone body concentrations interact to determine the level of glycolysis in neurons. Clinical management of blood glucose levels (as targeted directly in the low-glycemic index treatment [LGIT]; Pfeifer & Thiele 2005) does appear to play an important role in the efficacy of dietary treatment for epilepsy. Lowered glucose and modestly elevated ketone body concentrations (as seen in patients on the LGIT) may synergistically reduce glycolysis. Even without high ketone body concentrations, it is

possible that overall flux of ketone bodies (synthesis by liver and consumption by brain) is elevated. In addition, given a diet heavier in fats than in carbohydrates, it is possible that local ketogenesis by astrocytes (Cullingford et al. 2002) could play a role in changing the mix of fuels consumed by neurons in the brain.

The K_{ATP} – glycolysis hypothesis requires further exploration

Additional studies are needed both at the cellular level, to understand the mechanism of ketone body effects on glycolysis and K_{ATP} channels, and at higher levels (brain slices, animals, and people) to discern its role in the anticonvulsant mechanism of the KD. At the cellular level, we have recently developed a novel genetically-encoded fluorescent sensor of the intracellular ATP/ADP ratio (J. Berg and G. Yellen, unpublished). This tool, particularly when it is targeted to the submembrane compartment and elsewhere in neurons, should permit a more direct assessment of whether ATP compartmentation exists.

At higher levels, the most direct but also problematic approach would be to test the effect of K_{ATP} channel knockout on the efficacy of KD treatment in a mouse seizure model. The problem is that in every rodent seizure model tested, the efficacy of KD treatment has been extremely sensitive to mouse or rat strain, to age, and to the details of the diet and seizure model. The KD appears to be much less robust in treating rodent seizures than human seizures. Perhaps if it is possible to do genetic testing on a large enough cohort of human patients, then genetic contributions to efficacy – including, for instance, polymorphisms in K_{ATP} channel genes that affect the risk of diabetes, possibly due to their effect on channel open probability (Gloyn et al. 2004) – may provide additional clues to the actual mechanisms of protection in humans.

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